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# UNTERSUCHUNGEN ZUR PROPHYLAKTISCHEN UND THERAPEUTISCHEN BEEINFLUSSUNG DES STRESSULCUS DER RATTE

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# Role of Calcitonin in Stress Ulcer Formation of Various Rat Models. Preliminary Report

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*Summary:* During restraint stress (24 h) various rat models exhibit differences in ulcer index, a decrease in gastric perfusion ($pO_2$), serum gastrin and calcium, but increased pancreatic glucagon. Prophylaxis by calcitonin prevents gastrin from falling, improves markedly ulcer index in intact, sham-operated and thyroparathyroidectomized rats, is ineffective following parathyroidectomy and augments stress sensivity following thyroidectomy. It is suggested that, in the presence of exogenous calcitonin, both vulnerability of gastric mucosa and responsiveness of pancreatic alpha cells largely depend on concentration of extracellular calcium and availability of endogenous thyroidal calcitonin and parathyroid hormone.

*Key words:* rat models - stress ulcers - calcitonin prophylaxis - mucosal $pO_2$ - glucagon - gastrin - serum calcium - ulcer index

Exogenous calcitonin (CT) is said to diminish gastric secretion in humans (4, 11, 15) and animals (5, 9, 15) and to prevent stress induced gastroduodenal ulceration in rats (2, 8). Our previous findings (12, 13) of hyperglucagonemia, hypocalcemia and diminished gastric blood flow during restraint stress led us to assume that stress itself may be followed by a hitherto unknown sequence of hormonal events altered CT release included. Therefore we investigated whether CT plays a role during stress hypocalcemia in various rat models with or without endogenous CT sources. Furthermore, the response to exogenous CT of those exactly defined defect animals was evaluated with special emphasis to serum gastrin.

## ANIMALS AND METHODS

Randomized male Sprague-Dawley rats (SPF, 180 - 200 g b.w.), fed normal laboratory chow (C 1000, Altromin, Lage) were divided in 30 animals per group and left either intact or were prepared microsurgucally (pentobarbital-Na anesthesia) as following: intact; sham operated (sham); parathyroidectomized (PX); thyroidectomized (TX) and thyroparathyroidectomized (TPX) with oral replacement of thyroxine ( + $T_4$; 600 µg/l deionized drinking water). After 1 week each group was subdivided (n = 8 - 10) one third servind as controls. The remaining two thirds were either exposed to stress alone or received repeated

Table 1. *$pO_2$ of gastric mucosa, serum gastrin, thyroxine and total calcium ($Ca_t$), plasma glucagon and ulcer index (U.I) in various rat models without stress (controls), following 24 h stress (stress) and additional calcitonin (stress + CT) or vehicle (stress + V) administration. For symbols s. "animals and methods". Mean ± SEM; ( ): total number of rats/group and determinations/parameter unless otherwise indicated*

| | $pO_2$ mm Hg | Glucagon pg/ml | Gastrin pg/ml | $Ca_t$ mg/dl | Thyroxine µg/dl | ulcer index |
|---|---|---|---|---|---|---|
| controls | | | | | | |
| intact | 18.1 ± 0.8 | 69 ± 5 | 86 ± 11 | 9.60 ± 0.09 | 2.58 ± 0.28 (5) | o (9) |
| sham | 14.7 ± 0.2 | 100 ± 8 | 109 ± 28 | 9.77 ± 0.13 | – ± | o (9) |
| TX + $T_4$ | 15.0 ± 0.8 | 57 ± 8 | 78 ± 9 | 9.69 ± 0.09 | 2.66 ± 0.32 (6) | o (8) |
| PX | 12.2 ± 0.4 | 89 ± 13 | 104 ± 22 | 4.78 ± 0.19 | 2.21 ± 0.30 (6) | o (8) |
| TPX + $T_4$ | 12.1 ± 0.5 | 74 ± 20 | 75 ± 9 | 5.04 ± 0.23 | 2.99 ± 0.58 (6) | o (9) |
| stress | | | | | | |
| intact | 11.0 ± 0.6<br>< 0.001 | 336 ± 46<br>< 0.001 | 75 ± 3 | 8.79 ± 0.11<br>< 0.001 | 2.21 ± 0.30 (6) | 36 ± 8 (8) |
| sham | 10.5 ± 0.6<br>< 0.001 | 372 ± 98<br>< 0.05 | 53 ± 3<br>< 0.05 | 8.87 ± 0.04<br>< 0.001 | – | 30 ± 2 (8) |
| TK + $T_4$ | 10.8 ± 0.7<br>< 0.01 | 190 ± 52<br>< 0.05 | 61 ± 4 | 8.98 ± 0.14<br>< 0.001 | < 1.0 (5) | 25 ± 4 (8) |
| PX | 10.5 ± 0.4<br>< 0.01 | 246 ± 88<br>< 0.05 | 55 ± 3<br>< 0.05 | 4.97 ± 0.13 | 3.15 ± 0.40 (5) | 32 ± 2 (8) |
| TPX + $T_4$ | 8.5 ± 0.6<br>< 0.001 | 444 ± 132<br>< 0.05 | 73 ± 7 | 4.65 ± 0.20 | < 1.0 (6) | 60 ± 7 (9) |

| | | | | | | |
|---|---|---|---|---|---|---|
| stress + CT | | | | | | |
| intact | 11.3 ± 0.4 | 193 ± 62<br>0.05 | 103 ± 24 | 7.04 ± 0.21<br>< 0.001 | 1.98 ± 0.26 (6) | 15 ± 3 (9)<br>< 0.05 |
| sham | 11.5 ± 0.3 | 144 ± 19<br>< 0.05 | 86 ± 23 | 6.88 ± 0.14<br>< 0.001 | – | 17 ± 3 (9)<br>< 0.01 |
| TX + $T_4$ | 10.8 ± 0.8 | 697 ±141<br>< 0.01 | 77 ± 8 | 6.42 ± 0.14<br>< 0.001 | < 1.0 (5) | 44 ± 7 (8)<br>< 0.05 |
| PX | 10.4 ± 0.6 | 298 ± 40 | 79 ± 11<br>< 0.05 | 5.13 ± 0.30 | – | 36 ± 7 (9) |
| TPX + $T_4$ | 8.2 ± 0.7 | 674 ± 158 | 70 ± 5 | 4.49 ± 0.37 | 1.0 (6) | 24 ± 5 (8)<br>< 0.01 |
| stress + V | | | | | | |
| intact | 9.3 ± 0.3<br>< 0.05 | 396 ± 119 | 74 ± 5 | 8.93 ± 0.10 | – | 29 ± 6 (9) |

2-tailed t-test: control/stress and stress/stress + CT or V

CT injections (4 x 12 MRC units/kg)[1] sucutaneously during the stress period. Restraint stress (24 h) at room temperature was performed according to BRODIE and HANSON (3); partial oxygen pressure of gastric mucosa ($pO_2$) was monitored severalfold in fundic and antral regions exposed by laparotomy using the polarographic technique described earlier (12). Then ulcer index (U.I.) was calculated (10) and blood drawn from abdominal v. cava inf. Calcium was titrated by complexometry (EGTA, Fluka); gastrin and glucagon in serum and plasma resp. were measured by radioimmunoassay using highly specific antibodies: a) gastrin - raised in rabbits against 2-17 heptadecapeptide, final dilution 1:200 000, human gastrin I as standard reference and for iodination (Imperial Chemical Industries); no crossreaction with CCK-PZ ($ID_{50} > 10$ ng/ml) [2], glucagon or pure natural secretin; b) glucagon - rabbit 30 K[3] with minimum crossreactivity against gut glucagon extracts (1). Multiple dilutions of unknowns yielded results identical with appropriate points on standard curve. Intra- and interassay c.v. were 5 and 8 (gastrin), 10 and 14 per cent (glucagon) respectively. Restoration of plasma $T_4$ to normal range of this species following surgery was monitored by a modified CPB-assay using human alpha globulin (Koch-Light) as binder (7).

## RESULTS AND DISCUSSION

Table 1 demonstrates all relevant experimental data with the exception of $T_4$ being not measured in every group under study.With stable serum $T_4$, and a comparable concentration of gastrin and glucagon all control rats demonstrated zero ulcer index. However, animals subjected to surgery prior to the experiment and especially those animals with subsequent fall of serum calcium (PX, TPX) have lower baseline mucosal $pO_2$ than intact rats. Stress induced elevation of glucagon and a further decline of $pO_2$ can be demonstrated in all groups, whereas gastrin regularly falls slightly. Glucagon (=X) and $pO_2$ (=Y) are related by an inverse correlation ($n = 16$; $Y = 14.12 - 0.01 X$; $r = 0.75$; $p < 0.01$) supporting the assumption that this peptide might be involved in regulation of gastric blood perfusion rate (6, 13). Moreover, as can be shown with thyroidectomized rats (control vs. stress), also endogenous glucagon must be considered a CT-independent hypocalcemic hormone, unless release of other hypocalcemic substances during stress can be clearly documented. Conversely the lack of endogenous CT sources leads to an extremely high ulcer index with severe shock form gastric bleedings in TPX animals but not in rats merely devoid of thyroid (TX).

Exogenous CT administration during stress into thyroid intact rats either effects a remarkable reduction of ulcer index by appr. 50 per cent (intact, sham) or remains ineffective (PX). Following prior thyroidectomy the response

[1] Kindly donated by Drs. Rittel and Maier, Ciba-Geigy Corp. Basel, Switzerland

[2] inhibition dose: amount cholecystokinin-pancreozymin (on weight basis) needed for displacment of 50 per cent tracer initially bound to antibody;

[3] supplied by Dallas Diabetes Foundation, Dallas (USA).

to CT is not uniform: the most beneficial effect is observed in TPX rats, whereas TX rats paradoxically become more sensitive to stress. These differences in CT action upon ulcer index contrast to the uniformly lowered mucosal $pO_2$ previously incriminated by us for stimulating gastric stress ulceration (12, 13). Furthermore they cannot simply be the result of differences in CT mediated inhibition of stress induced glucagon release. Thus the causative factors for these differences in CT action must be recognized in the sometimes extremely low concentrations of extracellular calcium (TX, PX, TPX). These in turn lead to varying gradients of peri- and intracellular calcium dependent on either presence or absence of CT- and parathyroid hormone production sites. Accordingly, in rats devoid of thyroid (TX, TPX), pancreatic alpha cells may become more responsive to exogenous CT, but reduction of ulcer frequence by this agent obviously is restricted to animals with intact thyroid and parathyroids (intact, sham) or those lacking both (TPX).

Although serum gastrin during stress with CT prophylaxis instituted is slightly higher than during stress alone the widely varying ulcer index in the groups under study argues against acid theory of stress ulceration, i.e. deinhibition of antral G-cells due to diminished gastric acid secretion (2).

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LEBENSLAUF

Am 28. Juli 1951 wurde ich als Sohn des stellv. Sparkassenleiters Hans Steiner und seiner Ehefrau Hertha Steiner, geb. Rose, in Neustadt a. d. Waldnaab geboren.

Nach dem Besuch der Evang. Volksschule in Neustadt/WN und des Kepler-Gymnasiums in Weiden/Opf. legte ich am 2o. Juni 197o die Reifeprüfung ab.

Im Wintersemester 197o/71 begann ich in Erlangen mit dem Studium der Medizin. Den vorklinischen Teil schloß ich im März 1973 mit dem Physikum ab.

Am 28. April 1973 heiratete ich die Regierungsangestellte Frl. Margot Puckl aus Neustadt/WN.

Am 6. August 1976 legte ich das Staatsexamen mit der Note "sehr gut" ab.

Erlangen, im August 1976

Herbert Richard Steiner

( Herbert Richard Steiner )